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Claims

[c1] We claim:

A method of decreasing the expression of a target gene in a cell of a mammalian subject comprising administering to the subject in vivo a therapeutically effective amount of an RNAi expression cassette, comprising:

(a) providing a recombinant adeno-associated viral vector, wherein said vector comprises said RNAi expression cassette whose RNA expression product(s) directly or indirectly lead to the decrease of expression of an RNAi target gene, wherein the RNA expression product(s) of the RNAi expression cassette comprise a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the RNAi target gene mRNA transcript

(b) delivering said recombinant adeno-associated viral vector to and/ or within said mammalian subject wherein transduction of suitable target cells results in expression of said RNAi expression cassette.

[c2] A method of decreasing the expression of (at least) one target gene in a cell of a mammalian subject comprising administering to the subject in vivo a therapeutically ef-

fective amount of (at least) one RNAi expression cassette, comprising:

(a) providing (at least) one recombinant adeno-associated viral vector, wherein said vector comprises (at least) one RNAi expression cassette whose RNA expression product(s) directly or indirectly lead to the decrease of expression of an RNAi target gene, wherein the RNA expression product(s) of the RNAi expression cassette comprise a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the RNAi target gene mRNA transcript

(b) delivering said recombinant adeno-associated viral vector(s) to and/ or within said mammalian subject wherein transduction of suitable target cells results in expression of said RNAi expression cassette.

[c3] The method of claims 1 and 2, wherein expression of the RNA coding region of the RNAi expression cassette results in the down-regulation of the expression of the RNAi target gene, wherein the target gene comprises a sequence that is at least about 90% identical with the RNA coding region.

[c4] The method of claims 1 and 2, in which the RNAi target gene expression is inhibited by at least 10%.

[c5] The method of claims 1 and 2, wherein said RNAi expression cassette(s) encode one or more RNA molecules which are capable of forming an RNA interference inducing double-stranded RNA complex.

[c6] The method of claims 1 and 2, wherein said RNAi expression cassette encodes (at least) one RNA molecule which is self-complementary.

[c7] The method of claims 1 and 2, wherein said RNAi expression cassette encodes (at least) two separate complementary single-stranded RNA molecules.

[c8] The RNA molecule or RNA molecules of claims 6 and 7, wherein said RNA molecule or RNA molecules are capable of forming an RNA interference inducing double-stranded RNA complex.

[c9] The method of claims 1 and 2, wherein (at least) two recombinant adeno-associated viral vectors are used with each vector comprising its own RNAi expression cassette, and each RNAi expression cassette encoding at least one RNA molecule which is complementary to the RNA molecule expressed by the other RNAi expression cassette.

[c10] The RNA molecule(s) of claims 5, 6, 7, 8 and 9 having a nucleotide sequence which is substantially identical and/

or complementary to at least a part of the RNAi target gene.

[c11] The RNA molecule(s) of claims 5, 6, 7, 8 and 9 with the RNA molecule(s) being siRNA.

[c12] The method of claims 1 and 2, wherein said RNAi expression cassette encodes a self-complementary RNA molecule comprising a sense region, a loop region and an antisense region.

[c13] The method of claim 12, wherein the loop region is about 2 to about 10 nucleotides in length.

[c14] The method of claim 12, wherein the sense region and the antisense region are each between about 10 and about 30 nucleotides in length.

[c15] The method of claim 12, wherein the sense region hybridizes under stringent conditions to a nucleotide sequence of the RNAi target gene, and the antisense region, which is a complementary inverted repeat of said sense region, hybridizes to said sense region to form a hairpin structure.

[c16] The method of claims 1 and 2, wherein said RNAi expression cassette comprises a first promoter and a second promoter, each operably linked to an RNA coding

region, such that expression of the RNA coding region from the first promoter results in the synthesis of a first RNA molecule and expression of the RNA coding region from the second promoter results in the synthesis of a second RNA molecule substantially complementary to the first RNA molecule.

[c17] The method of claims 1 and 2, wherein said RNAi expression cassette comprises two promoters operably linked to the same RNA coding region, such that expression of the RNA coding region from the first promoter results in the synthesis of a first RNA molecule and expression of the RNA coding region from the second promoter results in the synthesis of a second RNA molecule substantially complementary to the first RNA molecule.

[c18] The method of claims 1 and 2, wherein said RNAi expression cassette encodes (at least) two RNA molecules, wherein

(a) one of the (at least) two RNA molecules consists essentially of a ribonucleotide sequence which corresponds to a nucleotide sequence of the RNAi target gene and another of the (at least) two RNA molecules consists essentially of a ribonucleotide sequence which is complementary to said nucleotide sequence of the RNAi target gene

(b) the (at least) two RNA molecules are separate

complementary strands that hybridize to each other to form a double-stranded RNA complex, and the double-stranded RNA complex directly or indirectly inhibits expression of the RNAi target gene.

[c19] The method of claims 1 and 2, wherein said RNAi expression cassette comprises a promoter operably linked to a DNA sequence which, when expressed by a host cell produces one RNA molecule having:

(a) homology to at least one target mRNA expressed by the host cell

(b) two (internally) complementary RNA regions

wherein the expressed RNA reduces the intracellular concentration of the target mRNA or any substantially similar endogenous mRNA either directly or indirectly.

[c20] The method of claims 1 and 2, wherein said RNAi expression cassette encodes (at least) one RNA molecule for inhibiting expression of a target gene, comprising a first nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the RNAi target gene, and a second nucleotide sequence which is a complementary inverted repeat of said first nucleotide sequence and hybridizes to said first nucleotide sequence to form a hairpin structure.

[c21] The RNA molecule of claim 19, wherein the two nu-

cleotide sequences are joined by an RNA loop structure.

[c22] The method of claims 1 and 2, wherein expression of said RNAi expression cassette leads to the generation of a double-stranded RNA complex comprising:

(a) a first RNA portion capable of hybridizing under physiological conditions to at least a part of an mRNA molecule encoded by a gene; and

(b) a second RNA portion wherein at least a part of the second RNA portion is capable of hybridizing under physiological conditions to the first RNA portion.

[c23] The RNA complex of claim 22 wherein the first and second portions are separate ribonucleic acid molecules.

[c24] The RNA complex of claim 22 wherein the first and second portions are comprised within the same RNA molecule.

[c25] The method of claims 1 and 2, wherein said RNAi expression cassette encodes a linear RNA molecule capable of forming a double-stranded RNA complex wherein the RNA molecule comprises:

(a) a first portion that hybridizes under physiologic conditions to at least a portion of an mRNA molecule encoded by a gene; and

(b) a second portion wherein at least part of the sec-

ond portion is capable of hybridizing to the first portion to form a hairpin double-stranded RNA complex.

[c26] The linear RNA molecule of claim 25 further comprising a third portion of ribonucleic acid interposed between the first and second portions.

[c27] The linear RNA molecule of claim 26 wherein the third portion promotes hybridization between the first and second portion.

[c28] The method of claims 1 and 2, wherein said RNAi expression cassette encodes a linear RNA molecule capable of forming a double-stranded RNA complex wherein the RNA molecule comprises:

(a) a first portion that comprises a region of RNA that is complementary to at least a portion of an mRNA molecule encoded by a gene

(b) a second portion capable of hybridizing to at least part of the first portion

(c) a third portion positioned between the first and second portions to facilitate the hybridization of the first and second portions with one another.

[c29] The linear RNA molecule of claim 22 and 25 wherein the second sequence comprises a transcription termination

signal positioned at the 3' end of the linear RNA molecule.

[c30] The method of claims 1 and 2, wherein the recombinant adeno-associated viral vector further comprises a gene of interest.

[c31] The method of claims 1 and 2, wherein the rAAV vector is of serotype 1, 2, 3, 4, 5, 6, 7 or 8 or any homologous serotypes or hybrids thereof.

[c32] The method of claims 1 and 2, wherein said RNAi expression cassette comprises an RNA Polymerase III promoter.

[c33] The method of claims 1 and 2, wherein said RNAi expression cassette comprises an RNA Polymerase II promoter.

[c34] The method of claims 1 and 2, wherein said RNAi expression cassette comprises an RNA Polymerase I promoter.

[c35] The method of claims 1 and 2, wherein said RNAi target gene causes or is likely to cause disease.

[c36] The method of claims 1 and 2, wherein said RNAi target genes are the Rhodopsin gene, the CCR5 gene, the CXCR4 gene, the VEGF gene, the HIF gene or any other

gene of therapeutic interest.

[c37] The method of claims 1 and 2, wherein said RNAi target gene is the Rhodopsin gene.

[c38] The method of claims 1 and 2, wherein said transduced cells are cells of and/or in the eye, retinal cells, retinal pigment epithelial cells, photoreceptor cells, cells of the eye, gut cells, muscle cells, lung cells, intestinal cells, liver cells, pancreatic cells, hematopoietic cells, stem cells, skin cells, endothelial cells, neurons, cells of ectodermal origin, cells of neurodermal original, cells of endodermal original and/ or brain cells.

[c39] The method of claims 1 and 2, wherein said transduced cells are photoreceptor cells.

[c40] The pharmaceutical preparation comprising a recombinant adeno-associated viral vector comprising an RNAi expression cassette as claimed in claim 1.

[c41] The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/ or administered by intravenous administration.

[c42] The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/ or administered by intra-arterial administration.

[c43] The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/ or administered by intracavity injection.

[c44] The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/ or administered by injection into tissue.

[c45] The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/ or administered by injection into gaps in tissue.

[c46] The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/ or administered by local administration.

[c47] The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/ or administered by inhalation and/ or nasal instillation.

[c48] The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/ or administered by intraocular and/ or intravitreal administration.

[c49] A method for treating a mammalian subject with an autosomal-dominant disorder or other disease including cancer and infectious diseases by administering to the subject an adeno-associated viral vector for initiating

decrease of RNAi target gene expression at the mRNA level, wherein the method comprises using RNAi to achieve post-transcriptional gene silencing.

[c50] The method of claim 49, wherein the mammalian subject is a human patient.